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ORIGINAL ARTICLE

CDC20 and its downstream genes: potential prognosis factors of osteosarcoma

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Abstract

Background We investigated the microarray data GSE42352 to identify genes that can be used as prognosis factors in osteosarcoma.

Methods Gene Ontology (GO) biological process analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of Cytoscape ClueGo were used in verifying the function of different genes. Realtime-PCR were used to confirm the microarray results. 83 patient samples were collected and underwent Kaplan–Meier survival analysis and multivariate analysis to predict the prospect of genes using as prognosis factors.

Results After analyzing the microarray data GSE42352, mitosis metaphase to anaphase-related genes CDC20, securin, cyclin A2 and cyclin B2 were found to be overexpressed in osteosarcoma cell lines. Kaplan–Meier survival analysis showed that overexpression of these genes can predict poor prognosis outcomes in osteosarcoma patients. Furthermore, any combination of the four genes seems to be more effective in predicting osteosarcoma outcomes than any of these genes alone.

Conclusions CDC20 and its downstream substracts securin, cyclin A2 and cyclin B2 are good factors that can predict prognosis outcomes in osteosarcoma. Any two combination of these four genes are more effective to be used as osteosarcoma prognosis factors.

Keywords Osteosarcoma · Cell cycle · CDC20 · Prognosis

Introduction

Osteosarcoma is a bone tumor that primarily occurs in adolescents and young adults. The incidence rate in 0- to 24-year-old people is approximately 4.4 per million each year [1, 2]. Patients are commonly treated with neoadjuvant chemotherapy, which includes 3–4 rounds of chemotherapy prior to surgery and approximately 12 rounds of chemotherapy after surgery. However, the 5-year survival rate is still low at approximately 60–70% [3]. The currently available prognosis factors are still not useful in predicting the treatment outcomes of patients. Discovering new prognosis factors that distinguish good therapeutic effects in patients will be meaningful.

The dysregulation of proteins during cell cycle may lead to many diseases, especially cancer. Thus, cell cycle-related proteins have become important therapeutic targets in cancer therapy. By analyzing the database GSE42352 [4], we found that cell division cycle 20 homologue (CDC20) and its downstream genes securin, cyclin A2 and cyclin B2

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were overexpressed in osteosarcoma cell lines. CDC20 is an important cell cycle regulator that binds to the APC/C complex during the metaphase-anaphase transition [5]. It activates the ubiquitin ligase activity of the APC/C complex, leading to the degradation of APC/C^{CDC20} substrates such as securin and cyclin A [6]. The APC/C^{CDC20} complex is also a regulator of the spindle checkpoint which ensures the proper separation of sister chromatids; if the sister chromatids are misaligned, then the cell cycle will be arrested [7]. The overexpression of CDC20 leads to cell mitosis accompanied by misaligned sister chromatids.

The targeting substrates of APC/C^{CDC20} include securin, cyclin A, and cyclin B, among others [8–10]. Securin is an inhibitor of anaphase that controls sister chromatid separation [11]. When cells pass through the spindle checkpoint, securin is then degraded by APC/C^{CDC20} and releases its inhibition of separase, which leads to sister chromatid separation [6]. It has been found that the expression of nondegradable securin leads to incomplete chromatid separation, although metaphase is not arrested [8]. Cyclin A2 plays important roles in the somatic cell proliferation cycle, especially in sister chromatid segregation [10]. The overexpression of cyclin A2 was discovered to delay the metaphase to anaphase transition [10]. Researchers have discovered that abnormal expression of cyclin B2 will lead to inaccurate centrosome separation and aberrant spindles, which will affect the accurate separation of sister chromatids [12]. Cyclin B2 controls centrosome separation antagonistically with tumor suppressor gene p53, which indicates that cyclin B2 may also play important roles in cancer initiation and cancer progression [12].

In this study, CDC20, securin, cyclin A2 and cyclin B2 were found to be overexpressed in osteosarcoma cell lines by analyzing the database GSE42352. We then focused on the relationship between these cell cycle-related genes with the clinical outcome of osteosarcoma by testing the patient tumor samples. Our study found that overexpression of CDC20, securin, cyclin A2 and cyclin B2 is correlated with poor prognosis of osteosarcoma, which indicates that these four genes can be used as prognostic factors of osteosarcoma in the future.

Materials and methods

Microarray analysis

Microarray data were downloaded from Series GSE42352, Gene Expression Omnibus (GEO), National Center for Biotechnology Information (NCBI), US National Library of Medicine. The data were divided into two groups: the osteoblast group (G1, which includes GSM825623-25) and the high-grade osteosarcoma cell line group (G2, which includes GSM1038236-54).

The differentiated gene Gene Ontology (GO) biological process network and KEGG network were analyzed using Cytoscape (National Resource for Network Biology, USA, Version 3.2.1) ClueGo (Version 2.3.5) [13]. The uniport IDs of genes were retrieved from https://www.uniprot.org/.

Cell cultures

All human osteosarcoma cell lines were obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) with 10% fetal bovine serum (FBS), 100 U/mL penicillin G, and 100 mg/mL streptomycin and were maintained at 37 °C in 5% CO_2. Cell line authentication was confirmed by using short tandem repeats profiling.

RNA extraction, reverse transcription

We used RNAprep Pure Cell/Bacteria Kit (DP430, TIANGEN) to extract cell RNA. PrimeScript™ RT Master Mix (Perfect Real Time) (RR036A, Takara) is used in cDNA synthesis. All steps were followed according to the manufacturer's instructions.

Real-time PCR

Real-time PCR was conducted by using TB Green™ Premix Ex Taq™ II (TliRNaseH Plus) (RR820A, Takara) in real-time PCR tubes (PCR-02-C, Axygen). The real-time PCR machine was a Biorad CFX 96touch. All relative quantification of targeted genes were normalized by using an endogenous control (GAPDH).

Primers

CDC20-forward: GCTTTGAACCTGAACGGTTTTG.
CDC20-reverse: TCTGGCGCATTTTGTGGTTTT.
Securin-forward: ACCCGTGTGGTTGCTAAGG.
Securin-reverse: ACGTGGTGTTGAAACTTGAGAT.
Cyclin A2-forward: GGATGGTAGTTTTGAGTCACCAC.
Cyclin A2-reverse: CACGAGGATAGCTCTCATACTGT.
Cyclin B2-forward: TGCTCTGCAAAATCGAGGACA.
Cyclin B2-reverse: GCCAATCCACTAGGATGGCA.
GAPDH-forward: ACAACTTTGGTATCGTGGAAGG.
GAPDH-reverse: GCCATCACGCCACAGTTTC.

Antibodies

The antibodies used for immunochemistry staining were as follows: CDC20 (ab215908, Abcam), securin (ab79546, Abcam), cyclin A2 (ab38, Abcam) and cyclin B2 (ab185622, Abcam).

Patients

Tumor samples of patients who received surgery were collected between January 2008 and December 2011 at the Department of Musculoskeletal Oncology, the First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China. The study was approved by institutional review board of the First Affiliated Hospital of Sun Yat-Sen University and the informed consent of patients was obtained before sample collection.

Immunochemistry staining

83 patient samples were collected, fixed with formalin, embedded in paraffin and sectioned. Hematoxylin–Eosin staining (HE staining) was used to confirm the osteosarcoma position. Immunochemistry staining was performed according to the manufacturer's instructions (K5007, Dako).

Statistical analysis

All data were analyzed by using IBM SPSS Statistics 21 and were presented as the mean ± SD. The differences between different groups was evaluated by using two-tailed *t* test or χ^2 test, and *P* value < 0.05 was considered as statistically significant. $P < 0.05$*; $P < 0.01$**; $P < 0.005$***. Cox proportional hazard model was used in the multivariate analysis.

Results

CDC20, securin, cyclin A2 and cyclin B2 were overexpressed in osteosarcoma

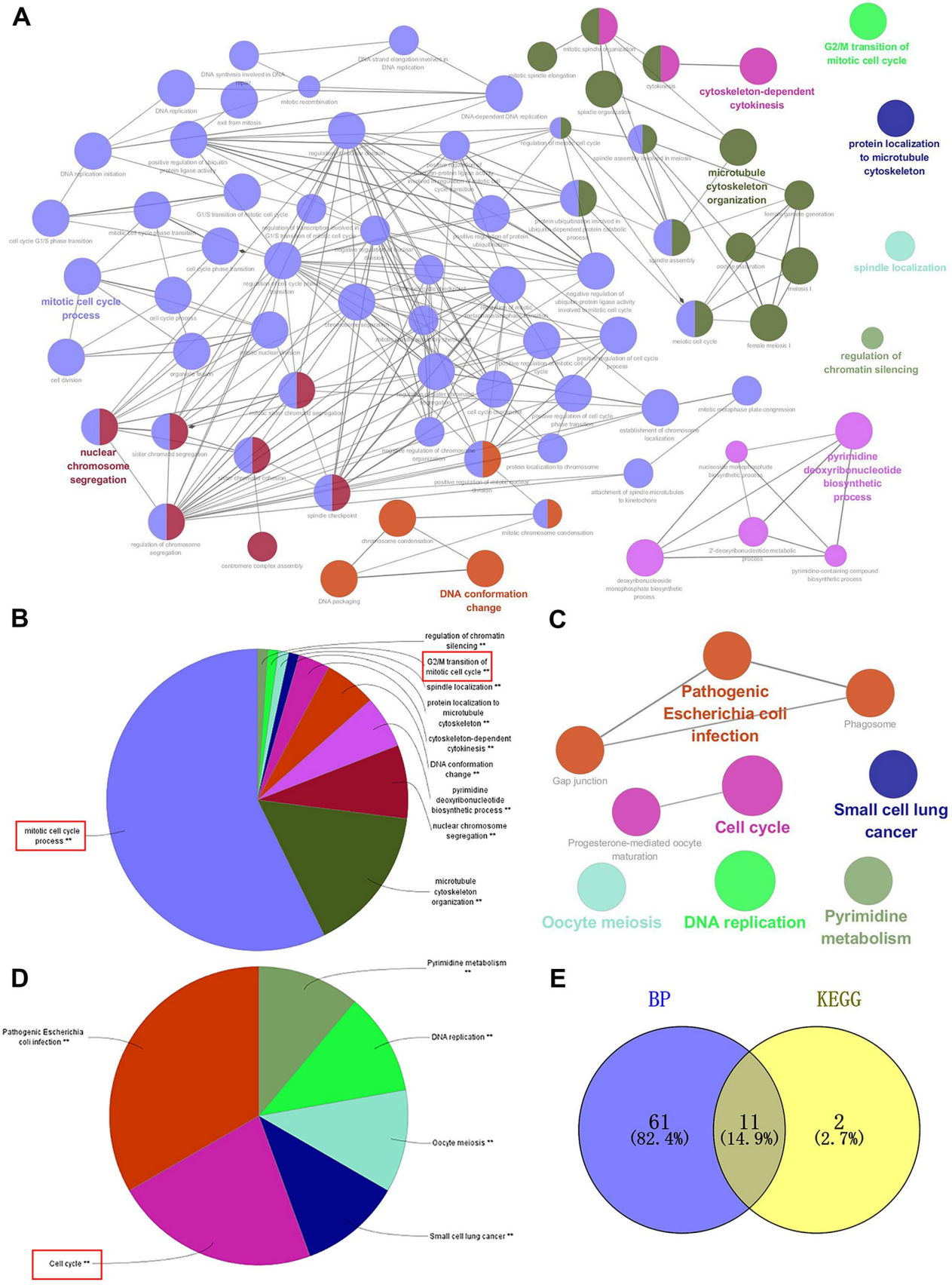
Microarray data GSE42352 were divided into two groups, namely, G1 (Osteoblast) and G2 (high-grade osteosarcoma cell line), and logFC (G2–G1) was calculated. In total, 155 genes whose logFCs (G2–G1) > 1.5 were obtained, and only 128 genes could be retrieved with a uniport ID (data not shown). After loading uniport IDs to Cytoscape ClueGo (show only pathways with pV ≤ 0.01), we obtained the GO biological process analysis results (Fig. 1a). All these genes were discovered to be involved in 10 different biological processes, including the mitotic cell cycle process and G2/M transition of the mitotic cell cycle (Fig. 1a, b; Supplemental Table 1). KEGG analysis (show only pathways with pV ≤ 0.01) also showed similar results; upregulated genes were associated with six pathways, i.e., cell cycle (Fig. 1c, d; Supplemental Table 2). These results showed that cell cycle pathways play very important roles in osteosarcoma.

We then investigated the cell cycle-related genes in GO biological process analysis and KEGG analysis and found that only 11 genes existed in both analyses (Figs. 1e, 2a; Supplemental Table 1, 2). By comparing the function, expression level and logFC (G2–G1) of these genes, we choose CDC20, securin, cyclin A2 and cyclin B2, which were overexpressed in osteosarcoma cell lines compared with osteoblast cell lines, for further study (Fig. 2a). mRNA expression levels of CDC20, securin, cyclin A2 and cyclin B2 in the human osteoblast cell line hFOB and in the osteosarcoma cell lines HOS, MG63, SAOS2, SJSA, and U2OS were then tested to verify the microarray results. Expression of all these four genes was approximately 2- to 4-fold higher in osteosarcoma cell lines than in the osteoblast cell line, and the more aggressive cell line SJSA had higher mRNA expression levels (Fig. 2b).

These results confirm that CDC20, securin, cyclin A2 and cyclin B2 were overexpressed in osteosarcoma cell lines, indicating that they may play important roles in osteosarcoma tumorigenesis and progression. Aggressive osteosarcoma cell lines seem to express higher levels of CDC20, securin, cyclin A2 and cyclin B2, suggesting that high expression of the four genes may related to poor prognosis.

CDC20, securin, cyclin A2 and cyclin B2 expression predicted osteosarcoma prognosis outcomes

We collected 83 patient surgery samples between January 2008 and December 2011, and the statistical data are shown in Table 1. Immunochemistry staining of CDC20, securin, cyclin A2 and cyclin B2 was performed on tissue sections of the tumors. CDC20 was mainly expressed in the nucleus in most patient samples; among them, 53 samples (63.9%) were regarded as positive, and 30 samples (36.1%) were regarded as negative (Table 2; Figs. 3a, 4a). Kaplan–Meier survival analysis was then applied to compare the survival time of the CDC20-positive group and the CDC20-negative group. We found that the CDC20-positive group had a poor prognosis outcome, whose 5-year overall survival rate was 34.0% (Table 2; Fig. 4a). Securin was expressed in both the nucleus and cytoplasm, Kaplan–Meier survival analysis showed that securin-positive patients seem to have poor prognosis outcomes, with a 25.5% 5-year overall survival rate in comparison with securin-negative patients (Table 2; Figs. 3b, 4b). Cyclin A2 was also expressed in both the nucleus and cytoplasm and cyclin A2-positive samples were related with poor prognosis outcomes along with a 26.5% 5-year overall survival rate (Table 2; Figs. 3c, 4c). Cyclin B2 was mainly expressed in the cytoplasm, cyclin B2-positive cases was associated with poor prognosis accompany by a 5-year overall survival rate of 27.3% (Table 2; Figs. 3d, 4d).


A
G2/M transition of mitotic cell cycle
cytoskeleton-dependent cytokinesis
protein localization to microtubule cytoskeleton
microtubule cytoskeleton organization
spindle localization
regulation of chromatin silencing
mitotic cell cycle process
nuclear chromosome segregation
pyrimidine deoxyribonucleotide biosynthetic process
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B
regulation of chromatin silencing **
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mitotic cell cycle process **
C
Pathogenic Escherichia coli infection
Phagosome
Gap junction
Cell cycle
Small cell lung cancer
Progesterone-mediated oocyte maturation
Oocyte meiosis
DNA replication
Pyrimidine metabolism
D
Pyrimidine metabolism **
DNA replication **
Oocyte meiosis **
Small cell lung cancer **
Cell cycle **
Pathogenic Escherichia coli infection **
E
BP
KEGG
61 (82.4%)
11 (14.9%)
2 (2.7%)

◂**Fig. 1** Gene analysis of microarray data GSE42352. **a** Network of the GO biological process analysis by using Cytoscape ClueGo (show only pathways with pV ≤ 0.01). **b** Specific gene cluster analysis of the GO biological process analysis. **c** Network of KEGG analysis by using Cytoscape ClueGo (show only pathways with pV ≤ 0.01). **d** Specific gene cluster analysis of the KEGG analysis. **e** Venn diagram shows 11 genes that existed in both the GO biological process analysis and the KEGG analysis

The correlation between metastasis rate and CDC20, securin, cyclin A2, cyclin B2 gene expressions were also detected. As shown in Table 3, CDC20 positive patients had a higher metastasis rate of 60.4% compare to CDC20 negative patients whose metastasis rate were 23.3%. Similarly, Securin positive patients, cyclin A2 positive patients and cyclin B2 positive patients also had a higher metastasis rate ranging from 76.6 to 82.4%, while related negative patients' metastasis rates were low (Table 3).

The results above showed that high expression of CDC20, securin, cyclin A2 and cyclin B2 was correlated with higher metastasis risk and poor prognosis outcomes in osteosarcoma.

Any combination of two of CDC20, securin, cyclin A2 and cyclin B2 are effective prognostic factors for osteosarcoma

By analyzing patient sample immunochemistry staining results, we discovered that 16 patient samples (19.3%) were

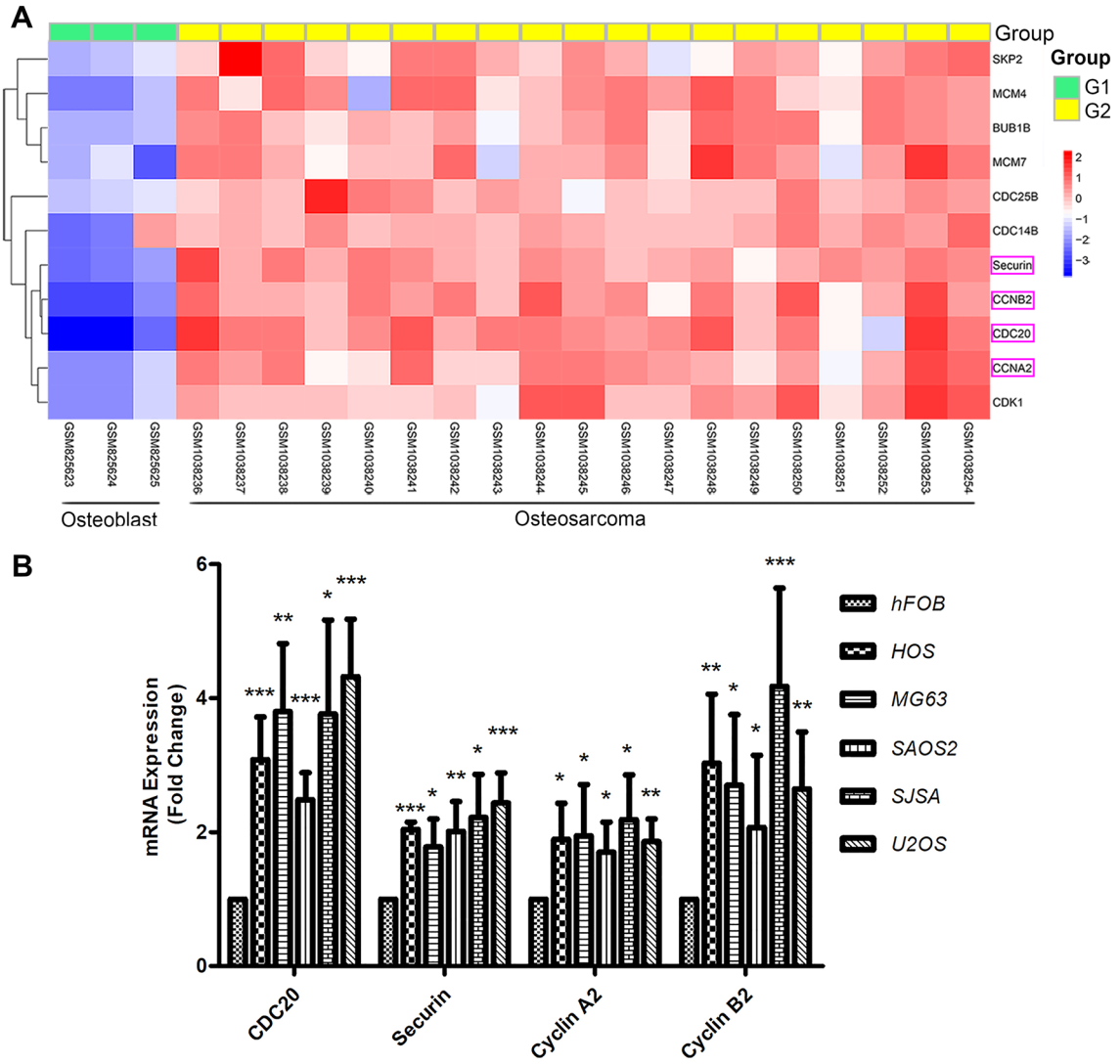

Fig. 2 CDC20, securin, cyclin A2 and cyclin B2 were overexpressed in osteosarcoma cell lines. **a** Heatmap of the above 11 genes that existed in both analyses of the microarray database GSE42352. **b** mRNA expression levels of CDC20, securin, cyclin A2 and cyclin B2 in human osteoblast cell line hFOB and in osteosarcoma cell lines HOS, MG63, SAOS2, SJSA, and U2OS. $P < 0.05$*; $P < 0.01$**; $P < 0.005$***

Table 1 Clinical characteristics of osteosarcoma patients

	Number of patients	Percentages (%)
Sex		
Male	40	48.2
Female	43	51.8
Age		
0–10	9	10.8
11–20	57	68.7
21–30	9	10.8
31 and above	8	9.6
Median	15	
Range	8–73	
Tumor stage		
I + II	44	53.0
III	39	47.0
Metastasis		
Yes	39	47.0
No	44	53.0
Location		
Distal femur	36	43.4
Proximal tibia	27	32.5
Proximal femur	3	3.6
Distal tibia	6	7.2
Humerus	9	10.8
Pelvis	2	2.4

all negative among the four genes, while 17 patient samples (20.5%), 10 patient samples (12.0%), 21 patient samples (25.3%) and 19 patient samples (22.9%) had one to four positive genes respectively (Table 4; Fig. 5a). Kaplan–Meier survival analysis revealed that patients who had two or more positively expressed genes seemed to have a worse prognosis compared to those patients with no positive or only one positive gene (Table 4; Fig. 5a).

The above results suggested that any combination of two genes of the four, CDC20, securin, cyclin A2 and cyclin B2, is a more effective prognostic factor for osteosarcoma than any one of those four genes alone. We then examined patient survival time to confirm this statement. Kaplan–Meier survival analysis shows that any combination of two of the four genes can predict the prognosis results (Table 5; Fig. 5b–g). The CDC20 + securin, Securin + cyclin A2, and Securin + cyclin B2 groups seem to be more significant predictors, with a 5-year overall survival rate of 22.2% to 24.3% (Table 5; Fig. 5b–g).

Multivariate analysis was also conducted to evaluate the importance of these genes in predicting osteosarcoma prognosis. By examining 6 factors (CDC20, securin, cyclin A2, cyclin B2, positive gene numbers and metastasis) using Cox proportional hazard model, we found that metastasis showed the highest hazard ratio of 7.229 (Table 6). CDC20, securin, cyclin A2, cyclin B2 solely was unable to predict osteosarcoma outcome, while positive gene numbers can predict osteosarcoma prognosis independently (Table 6). The hazard ratio of two or more positive genes were 3.853, 4.466 and 4.905 (Table 6).

Above result showed that any combination of two of CDC20, securin, cyclin A2 and cyclin B2 was better prognosis factors for osteosarcoma.

Discussion

Osteosarcoma is the most common primary malignant bone tumor in childhood and adolescence. The clinical outcome of patients with osteosarcoma can be improved with chemotherapy, and the 5-year survival rate has reached 60–70%. MTX, ADM (cell cycle specific agents), DDP and IFO (cell cycle non-specific agents) are the four primary drugs used for Osteosarcoma chemotherapy. As known, high-dose and multi-drug neoadjuvant chemotherapy has been the gold standard for Osteosarcoma treatment, which remarkably increased good response rates and consequent improvement in the survival rates.

However, there are still no effective prognostic factors that can be helpful for clinical doctors in selecting treatments and predicting patient outcomes for osteosarcoma. The commonly used prognostic factors include: primary tumor site,

Table 2 Prognostic analysis of CDC20, securin, cyclin A2 and cyclin B2 genes in osteosarcoma

Gene expression	Number of patients	Percentages (%)	5-year overall survival rate (%)	*P* value
CDC20 positive	53	63.9	34.0	0.0285
CDC20 negative	30	36.1	56.7	
Securin positive	47	56.6	25.5	0.0001
Securin negative	36	43.4	63.9	
Cyclin A2 positive	34	41.0	26.5	0.0054
Cyclin A2 negative	49	59.0	53.1	
Cyclin B2 positive	44	53.0	27.3	0.0018
Cyclin B2 negative	39	47.0	59.0	

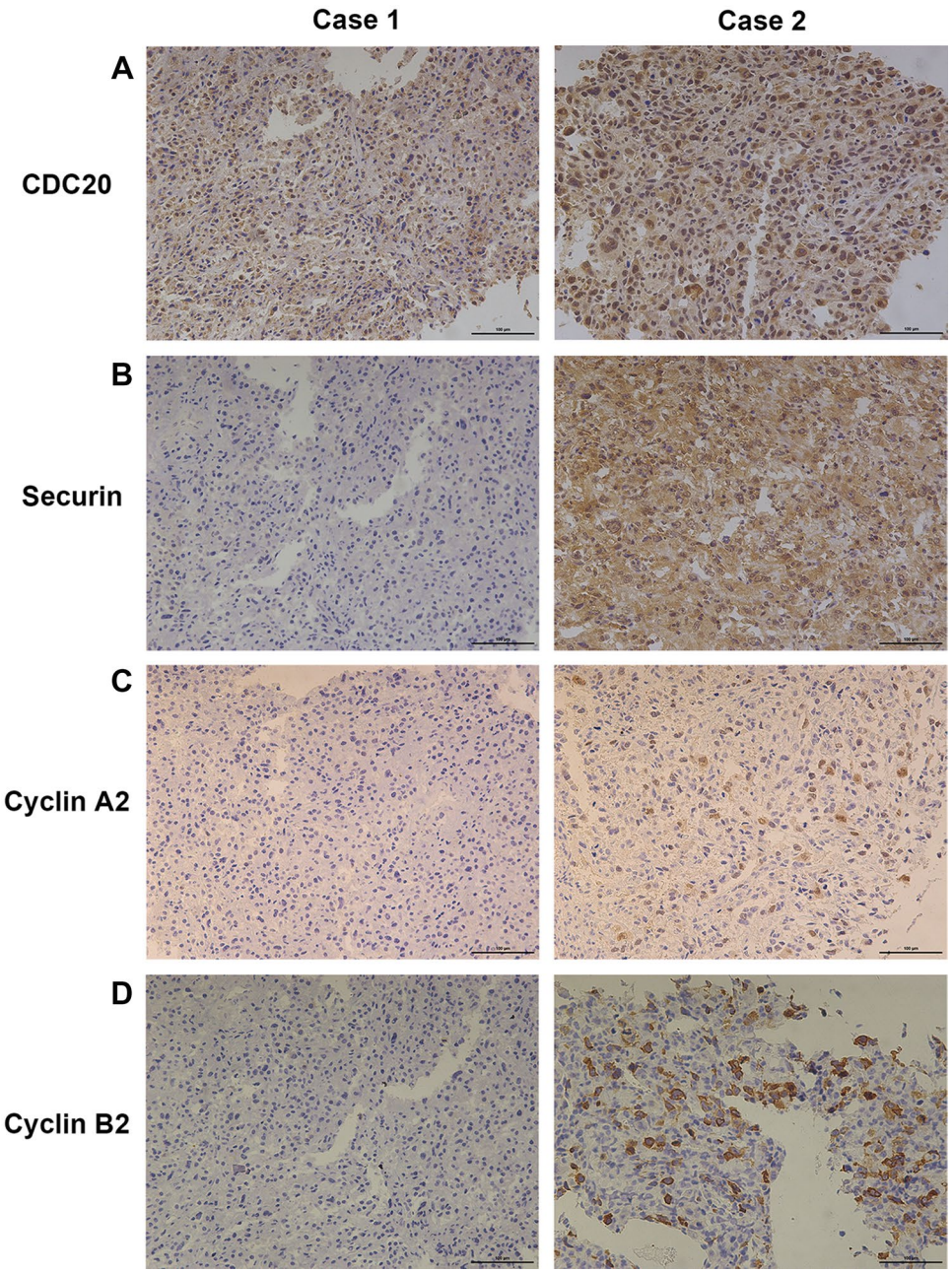

Fig. 3 Immunochemistry staining of CDC20, securin, cyclin A2 and cyclin B2 in osteosarcoma patient samples.

primary tumor size, metastasis or not, age, tumor grade, and initial treatment, among others [1, 14, 15]. Other factors, such as HER-2/neu, RB, p53, and p-glycoprotein expression, have been identified as osteosarcoma prognostic factors, but there have been no studies with large study populations [16–22]. In our study, we analyzed database GSE42352 using GO biological process analysis and KEGG analysis, and we discovered that cell cycle-related genes changed the most. This finding reinforces the idea that cell cycle-related genes must play important roles in osteosarcoma. Later, we discovered that eleven genes were present in both above analyses. By comparing the gene expression heatmap and the gene functions, we found that CDC20, securin, cyclin A2 and cyclin B2 are all involved in the mitosis metaphase to anaphase transition; thus, we chose these four genes for further study. Real-time PCR of an osteoblast cell line and several osteosarcoma cell lines confirmed the finding and we also discovered that more aggressive osteosarcoma cell lines had a higher expression level of the above four genes, suggesting that the four genes may be related to osteosarcoma progression.

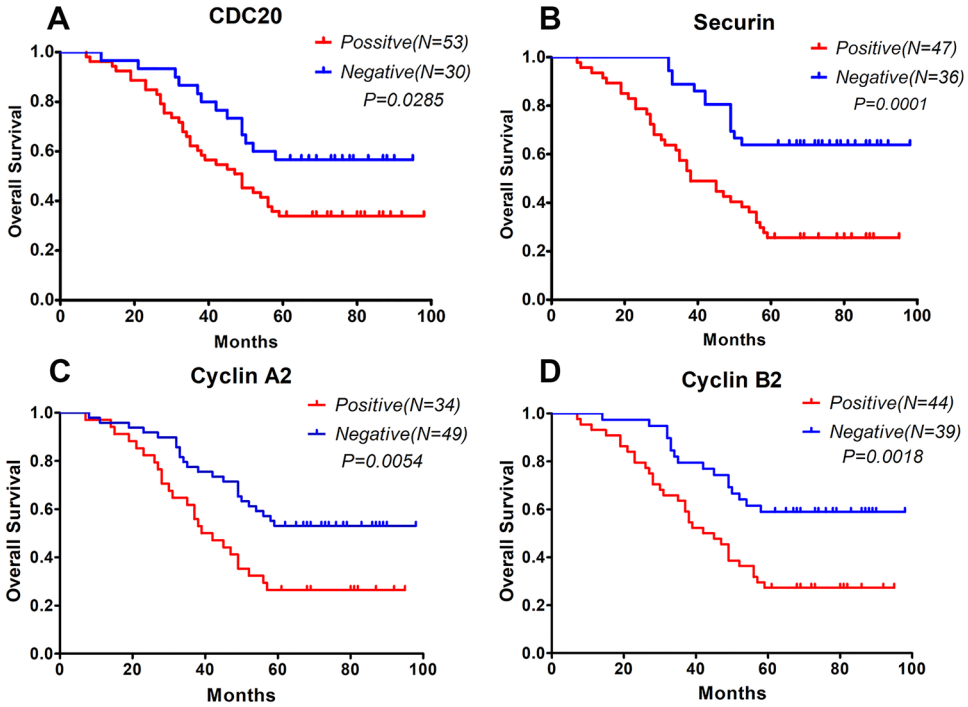

Fig. 4 Kaplan–Meier survival analysis of CDC20, securin, cyclin A2 and cyclin B2. **a** Kaplan–Meier survival analysis of CDC20. $P = 0.0285$. **b** Kaplan–Meier survival analysis of securin. $P = 0.0001$. **c** Kaplan–Meier survival analysis of cyclin A2. $P = 0.0054$. **d** Kaplan–Meier survival analysis of cyclin B2. $P = 0.0018$

Table 3 Correlation between metastasis rate and CDC20, securin, cyclin A2, cyclin B2 gene expressions in osteosarcoma

Gene expression	Metastasis rate (%)	*P* value
CDC20 positive	60.4	0.001
CDC20 negative	23.3	
Securin positive	76.6	0.000
Securin negative	8.3	
Cyclin A2 positive	82.4	0.000
Cyclin A2 negative	22.4	
Cyclin B2 positive	79.5	0.000
Cyclin B2 negative	10.3	

CDC20 is a vital cell cycle regulator that is conserved in most organisms. The most important function of CDC20 is to activate the APC/C complex, which controls the metaphase to anaphase transition in mitosis [5]. CDC20 had been found to regulate proliferation and invasion in osteosarcoma cells by regulating Bim and p21 [23, 24]. Furthermore, APC/C^{cdh} and APC/C^{cdc20} were also found to regulate BRD7 degradation which would inhibit cell proliferation in ostrosarcoma [25]. All these founding demonstrated that CDC20 play critical roles in osteosarcoma, but whether CDC20 and the substrates can predict osteosarcoma prognosis is still unknown.

Securin, cyclin A2 and cyclin B2 are substrates of CDC20. While the metaphase-anaphase transition initiated, CDC20 binds to APC/C complex and activated its ubiquitin ligase activity, which will lead to the degradation of securin, cyclin A2 and cyclin B2 [5, 6, 26]. Degradation of securin and cyclin A2 can activate the sister chromatid segregation while degradation of cyclin B2 can initiate centrosome separation [6, 10–12]. All these steps are necessary for metaphase-anaphase transition, and abnormal of these genes may lead to misaligned sister chromatid and the origin of cancer [8, 10, 12, 27]. Previous study has demonstrated that the metaphase arrest is eventually

Table 4 Relationship between positive gene numbers and osteosarcoma prognosis

Number of positive genes	Number of patients	Percentages (%)	5-year overall survival rate (%)	*P* value compare with no positive group	*P* value compare with 1 positive group
0	16	19.3	75.0	–	–
1	17	20.5	64.7	–	–
2	10	12.0	20.0	0.0045	0.0221
3	21	25.3	23.8	0.0018	0.0094
4	19	22.9	26.3	0.0010	0.0035

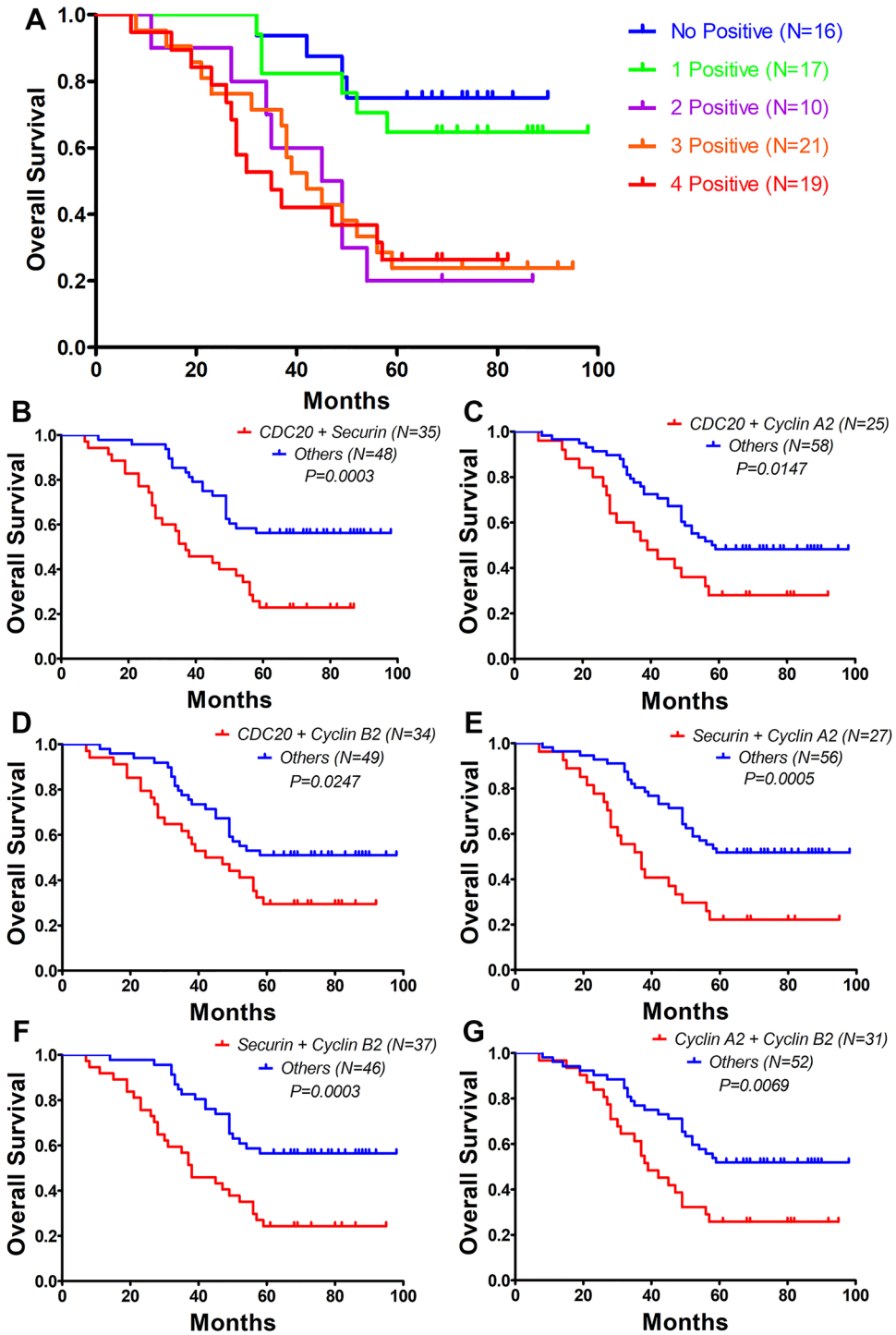

Fig. 5 Kaplan–Meier survival analysis of different gene combinations. **a** Kaplan–Meier survival analysis of patients with different positive gene numbers. **b** Kaplan–Meier survival analysis of CDC20 + securin coexpressed patients. $P = 0.0003$. **c** Kaplan–Meier survival analysis of CDC20 + cyclin A2 coexpressed patients. $P = 0.0147$. **d** Kaplan–Meier survival analysis of CDC20 + cyclin B2 coexpressed patients. $P = 0.0247$. **e** Kaplan–Meier survival analysis of securin + cyclin A2 coexpressed patients. $P = 0.0005$. **f** Kaplan–Meier survival analysis of securin + cyclinB2 coexpressed patients. $P = 0.0003$. **g** Kaplan–Meier survival analysis of cyclin A2 + cyclin B2 coexpressed patients. $P = 0.0069$

able to trigger cell death by the activation of apoptotic pathways [28]. Our study also confirmed that CDC20 and its downstream substrates securin, cyclin A2, and cyclin B2 plays important roles in osteosarcoma and can be used as biomarkers in predicting the prognosis outcomes of patients, which also suggest that the genes involved in cell cycle metaphase to anaphase transition play key roles in osteosarcoma. Because the expressions of CDC20, securin, cyclin A2 and cyclin B2 were not always consistent in osteosarcoma patient samples (Table 4; Fig. 5a). Besides, in breast cancer combination of CDC20 and securin immunoexpression was particularly strong in Kaplan–Meier analysis modelling short-term survival [29]. And in some cells, Cyclin A2 and cyclin B2 can be regulated in CDC20-independent [30, 31]. Therefore, we combined CDC20, securin, cyclin A2 and cyclin B2 by

Table 5 Gene combinations and their osteosarcoma prognostic analysis

Gene combination	Number of patients	Percentages (%)	5-year overall survival rate (%)	*P* value
CDC20 + securin	35	42.2	22.9	0.0003
Others	48	57.8	56.3	
CDC20 + cyclin A2	25	30.1	28.0	0.0147
Others	58	69.9	48.3	
CDC20 + cyclin B2	34	41.0	29.4	0.0247
Others	49	59.0	51.0	
Securin + cyclin A2	27	32.5	22.2	0.0005
Others	56	67.5	51.8	
Securin + cyclin B2	37	44.6	24.3	0.0003
Others	46	55.4	56.5	
Cyclin A2 + cyclin B2	31	37.3	25.8	0.0069
Others	52	62.7	51.9	

multivariate analysis for accurate prognosis analysis, and we discovered that using any combination of two of these four genes is more effective at predicting osteosarcoma prognosis outcomes which provides the more convincing prognosis index.

Though the current treatment of osteosarcoma is improved compared to previous treatment regimens, the therapeutic effect is still not good enough. Discovering efficient targeted therapies is a priority. Bevacizumab, a monoclonal antibody against vascular endothelial growth factor (VEGF) can inhibit osteosarcoma but will also lead to wound complications [32]. Other drugs such as trichostatin A, dasatinib and imatinib mesylate have also been found to inhibit osteosarcoma growth [33–35]. Our study shows that CDC20, securin, cyclin A2 and cyclin B2 are overexpressed in osteosarcoma cell lines and that high expression of these genes is correlated with poor prognosis. These findings also tell us that discovering new drugs that target these four genes, especially the metaphase to anaphase transition of cell cycle will be beneficial.

Table 6 Multivariate analysis of factors that affects osteosarcoma prognosis

Factor	Hazard ratio	95% CI	*P* value
Metastasis	7.229	2.360–22.145	0.001
CDC20	0.872	0.413–1.839	0.719
Securin	1.077	0.437–2.659	0.871
Cyclin A2	0.756	0.328–1.744	0.512
Cyclin B2	0.813	0.310–2.136	0.675
Positive genes (0)	1		
Positive genes (1)	1.485	0.419–5.262	0.540
Positive genes (2)	3.853	1.126–13.192	0.032
Positive genes (3)	4.466	1.499–13.311	0.007
Positive genes (4)	4.905	1.610–14.945	0.005

However, there are also some limitations in this study, such as the small number of patient samples. Whether these markers can help clinicians choose different treatments is unclear. Larger clinical studies are needed to confirm the results and help distinguish different patients so that they can be treated with different treatments to achieved better therapeutic effects. Furthermore, we have not focused on other pathways from the results available in database GSE42352. We may be able to can find more useful genes that are critical for osteosarcoma from the database.

In conclusion, by analyzing the microarray data GSE42352, we discovered that genes that are involved in the mitosis metaphase to anaphase transition, i.e., CDC20, securin, cyclin A2 and cyclin B2, are good predictors of prognosis outcomes in osteosarcoma. Moreover, securin, cyclin A2 and cyclin B2 are all substrates of the APC/C^{CDC20} complex, indicating an important role of CDC20 and the APC/C^{CDC20} complex in osteosarcoma progression. Our studies will provide evidence of better therapeutic targets that will predict prognosis outcomes for osteosarcoma in the future. Besides, we discovered that using any combination of two of these four genes is more effective at predicting osteosarcoma prognosis outcomes by multivariate analysis, which provides the more convincing prognosis index, which seems to be consistent with the multi-drug combination treatment.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

References

1. Osteosarcoma and Malignant Fibrous Histiocytoma of Bone Treatment (PDQ(R)) (2002) Health Professional Version, in PDQ Cancer Information Summaries. Bethesda (MD).
2. Mirabello L, Troisi RJ, Savage SA (2009) Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. Cancer 115(7):1531–1543
3. Isakoff MS et al (2015) Osteosarcoma: current treatment and a collaborative pathway to success. J Clin Oncol 33(27):3029–3035
4. Kuijjer ML et al (2013) IR/IGF1R signaling as potential target for treatment of high-grade osteosarcoma. BMC Cancer 13:245
5. McLean JR et al (2011) State of the APC/C: organization, function, and structure. Crit Rev Biochem Mol Biol 46(2):118–136
6. Stemmann O et al (2001) Dual inhibition of sister chromatid separation at metaphase. Cell 107(6):715–726
7. Bharadwaj R, Yu H (2004) The spindle checkpoint, aneuploidy, and cancer. Oncogene 23(11):2016–2027
8. Zur A, Brandeis M (2001) Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. EMBO J 20(4):792–801
9. Shirayama M et al (1999) APC(Cdc20) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. Nature 402(6758):203–207
10. Geley S et al (2001) Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. J Cell Biol 153(1):137–148
11. Nasmyth K (2002) Segregating sister genomes: the molecular biology of chromosome separation. Science 297(5581):559–565
12. Nam HJ, van Deursen JM (2014) Cyclin B2 and p53 control proper timing of centrosome separation. Nat Cell Biol 16(6):538–549
13. Shannon P et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504
14. Pakos EE et al (2009) Prognostic factors and outcomes for osteosarcoma: an international collaboration. Eur J Cancer 45(13):2367–2375
15. Collins M et al (2013) Benefits and adverse events in younger versus older patients receiving neoadjuvant chemotherapy for osteosarcoma: findings from a meta-analysis. J Clin Oncol 31(18):2303–2312
16. Gorlick R et al (1999) Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 17(9):2781–2788
17. Onda M et al (1996) ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 77(1):71–78
18. Kilpatrick SE et al (2001) Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod Pathol 14(12):1277–1283
19. Feugeas O et al (1996) Loss of heterozygosity of the RB gene is a poor prognostic factor in patients with osteosarcoma. J Clin Oncol 14(2):467–472
20. Heinsohn S et al (2007) Determination of the prognostic value of loss of heterozygosity at the retinoblastoma gene in osteosarcoma. Int J Oncol 30(5):1205–1214
21. Goto A et al (1998) Association of loss of heterozygosity at the p53 locus with chemoresistance in osteosarcomas. Jpn J Cancer Res 89(5):539–547
22. Serra M et al (2006) May *P*-glycoprotein status be used to stratify high-grade osteosarcoma patients? Results from the Italian/Scandinavian Sarcoma Group 1 treatment protocol. Int J Oncol 29(6):1459–1468
23. Shang G, Ma X, Lv G (2018) Cell division cycle 20 promotes cell proliferation and invasion and inhibits apoptosis in osteosarcoma cells. Cell Cycle 17(1):43–52
24. Gao Y et al (2018) Cdc20 inhibitor apcin inhibits the growth and invasion of osteosarcoma cells. Oncol Rep 40(2):841–848
25. Hu K et al (2014) Targeting the anaphase-promoting complex/cyclosome (APC/C)- bromodomain containing 7 (BRD7) pathway for human osteosarcoma. Oncotarget 5(10):3088–3100
26. Yu H (2007) Cdc20: a WD40 activator for a cell cycle degradation machine. Mol Cell 27(1):3–16
27. Ouellet V et al (2006) Tissue array analysis of expression microarray candidates identifies markers associated with tumor grade and outcome in serous epithelial ovarian cancer. Int J Cancer 119(3):599–607
28. Penas C, Ramachandran V, Ayad NG (2011) The APC/C ubiquitin ligase: from cell biology to tumorigenesis. Front Oncol 1:60
29. Karra H et al (2014) Cdc20 and securin overexpression predict short-term breast cancer survival. Br J Cancer 110(12):2905–2913
30. Shekhar R et al (2019) The microRNAs miR-449a and miR-424 suppress osteosarcoma by targeting cyclin A2 expression. J Biol Chem 294(12):4381–4400
31. Tschop K, Engeland K (2007) Cell cycle-dependent transcription of cyclin B2 is influenced by DNA methylation but is independent of methylation in the CDE and CHR elements. FEBS J 274(20):5235–5249
32. Navid F et al (2017) A phase II trial evaluating the feasibility of adding bevacizumab to standard osteosarcoma therapy. Int J Cancer 141(7):1469–1477
33. Deng Z et al (2016) Histone deacetylase inhibitor trichostatin a promotes the apoptosis of osteosarcoma cells through p53 signaling pathway activation. Int J Biol Sci 12(11):1298–1308
34. Shor AC et al (2007) Dasatinib inhibits migration and invasion in diverse human sarcoma cell lines and induces apoptosis in bone sarcoma cells dependent on SRC kinase for survival. Cancer Res 67(6):2800–2808
35. Gobin B et al (2014) Imatinib mesylate exerts anti-proliferative effects on osteosarcoma cells and inhibits the tumour growth in immunocompetent murine models. PLoS One 9(3):e90795